

ENVIRONMENTAL ANTIBIOTIC RESISTANCE: MONITORING IN BACTERIA FROM AVIFAUNA

Gionechetti F., 3^o di corso del Dottorato in Farmacologia, Chemioterapia e Microbiologia, XV ciclo. Durata del Dottorato in anni: 3. Sede di servizio: Università degli Studi di Trieste - Dipartimento di Scienze Biomediche - Via L.Giorgieri 9-11, 34127 TRIESTE.

Development of antibiotic resistance in bacteria is mainly due to the presence of resistance genes and to the selective pressure exerted by antibiotic use. Resistance can result from mutations of a resident genes or acquisition of additional genes coding for a resistance mechanism.

Antibacterial agents are used in agriculture techniques, in human and veterinary therapy and prevention of bacteria infections, and they are added continuously to animal feeds to promote growth. As a result of exposure to antibiotics, the level of resistance against antibiotics among bacteria belonging to the normal intestinal flora of human and animals increases. These bacteria not only constitute an enormous reservoir of resistance genes for potentially pathogenic bacteria, but also their level of resistance is considered to be a good indicator for the selection pressure exerted by antibiotic use in that population and for the resistance problems to be expected in pathogens. Indeed, reservoirs of antibiotic resistance in humans and in animals can interact in different ways: food and water are the most probable vectors of transmission to the intestinal flora.

Population of gulls (*Larus cachinnans michahellis*) has increased in nord-est of Italy during the past decade. This increase has been attributed to the ability of gulls to adapt to urban areas, infact they are able to nest on roofs and feed of urban waste. But gulls ability to over fly large areas suggests that their feces may have a potential role in dissemination of human disease and more underhand antibiotic resistance in urban and rural environment.

Transfer of antibiotic resistance genes between different species of bacteria can be facilitated by mobile DNA elements, such us transposons and plasmids. Integrons have been identified on these mobile elements, and they often contain one or more genes that encode antibiotic resistance. Nine classes of integrases have been described, but class 1 appears to be prevalent in *Enterobacteriaceae*.

In this work 102 cloacal swabs of *Larus cachinnans michahellis* were collected in natural reserve during spring in 2000 and 2001. There were isolated 214 *Enterobacteriaceae*, identified as *Proteus* sp.(n = 89), *E.coli* (n = 84), *Klebsiella* sp.(n = 18), *Salmonella* sp. (n = 17) and *Citrobacter* sp. (n = 6). Als o 162 Gram-positive bacteria were identified as *Enterococcus faecalis* (n = 101) and *Staphylococcus* sp. (n = 61).

Isolated avian strains were tested for susceptibility to a battery of antibiotics representing various classes of them. Gram-positive isolates showed low levels of resistance, but *Enterobacteriaceae* were resistant to a lot of antibiotics, especially to ampicillin (41% in *Salmonella*, 39% in *E.coli* and 23% in *Proteus*), to tetracycline (41% in *Salmonella*, 31% in *E.coli*), to streptomycin (55% in *Proteus*, 45% in *E.coli*, 39% in *Klebsiella* and 29% in *Salmonella*), to nalidixic acid (38% in *Proteus* and 22% in *E.coli*).

The high resistance levels found in Gram-negative strains are very important if we consider the natural habitat of monitorated avifauna, and we could explain this fact as a result of the spread of environmental antibiotic contaminants with their consequent selection pressure and the dissemination of antibiotic resistance genes by horizontal transfer. Gram-negative avian strains were also screened for class 1 integrase using a specific probes which hybridizes to conserved regions of integron encoded gene *intI1*. 25 of the 214 isolates were positive and showed to have class 1 integrons. Their sizes were detected by PCR with specific primers 5'CS-3'CS, flanking variable region of integron. Integrons ranged from 500 bp to 4800 bp.

The incidence of class 1 integrons were correlated with multiple-drug resistances exhibited by avian *Enterobacteriaceae* to streptomycin, trimethoprim-sulfamethoxazolo, tetracycline and chloramphenicol. At least integrons from avifauna were compared with integrons of uman clinical origin finding some homologies each other.